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GENETIC VARIATION AND SYSTEMATICS OF FOUR TAXA OF NEOTROPICAL WALKING STICKS (PHASMATODEA: PHASMATIDAE)

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Abstract.—Electrophoretically detectable genetic variation for six isozymes encoded by seven loci was analyzed in four taxa of walking sticks (Phasmatidae) that occur in a neotropical rainforest in eastern Puerto Rico. No phylogenetic analysis previously has been conducted on any phasmatid. All seven loci exhibited variation among *Diapherodes achalus, Lamponius portoricensis, Pseudobacteria yersiniana,* and an unnamed taxon (species X). Coefficients of genetic distance between these four taxa ranged from 0.349 to 0.571. The UPGMA of Rogers' genetic distance indicated considerable genic dissimilarity among taxa (the two taxa which were least dissimilar connected at a value of 0.350). The four taxa represent a holophyletic group for which an outgroup was not analyzed, this situation, in conjunction with the short internode distance in the Fitch-Margoliash analysis, provides only limited resolution of the phylogenetic associations among the four taxa.

Key Words: electrophoresis, isozymes, Puerto Rico, *Diapherodes, Lamponius, Pseudobacteria.*

The Phasmatidae, or walking sticks, are primarily a tropical group of folivores that occasionally have an economic impact on human-manipulated systems (Campbell 1960, 1961, 1966, 1974, Campbell and Hadlington 1967, Mananec 1966, 1967, 1968, Paine 1968). Most phasmatids are active nocturnally, and remain inactive on the surface of leaves and stems, or in leaf litter, during the day. Even at night, walking sticks are usually immobile and cryptic (Cott 1940, Moxey 1972). Besides these few cursory observations, the natural history of the Phasmatidae is poorly known. The best contemporary review of their biology was presented by Bedford (1978); however, his review primarily focused on Old World taxa. Although many studies have been conducted on the taxonomy of the Phasmatidae (see Bedford 1978), a dearth of information is available concerning their phylogenetic relationships. Detailed studies in the New World have lagged far behind those in the Old World.

Moxey (1972), in a detailed but yet unpublished study concerning systematics of walking sticks from the West Indies, recognized 54 Antillean species, distributed into 16 genera. Many of these species are restricted to one or only a few islands. Other than the work by Moxey (1971, 1972), the most recent research on phasmatids of the West Indies focused on population dynamics and natural history of *Lamponius por-*

toricensis from the Tabonuco rainforest of Puerto Rico (Willig et al. 1986). We presently are conducting studies aimed at examining the genetic bases of food preference and spatial distribution in *L. portoricensis*. The only other published study on Puerto Rican walking sticks examined the karyotypes in a selected few species and evaluated their response to low-level gamma irradiation (Virkki 1970). No phylogenetic analysis has been attempted on the Phasmatidae.

Puerto Rico and nearby Mona Island are inhabited by 11 species of walking sticks representing six genera (Moxey 1972). Of these 11 species, eight are endemic to Puerto Rico, two are found only on Puerto Rico and St. Thomas, and one is restricted to Mona Island. Of the taxa that we examined from eastern Puerto Rico, *D. achalus*, *L. portoricensis*, and *P. yersiniana* have widespread distributions throughout the mountainous regions of Puerto Rico. The fourth taxon is unnamed, and taxonomic work is being pursued by Garrison and Willig (pers. comm.). Although this unnamed taxon shares some morphological characteristics with the genus *Lamponius*, Moxey's (1972) morphological treatment of the West Indian taxa was not based upon features of the male genitalia (the primary characteristics used in systematic studies of the Orthoptera) and may represent spurious results. We do not formally classify this taxon and refer to it hereafter as species X. Species X occurs primarily in the mossy dwarf-forests (above 1000 m) of the Caribbean National Forest, located in the Luquillo Mountains on the eastern part of the island. The purpose of this project was to examine, using protein electrophoresis, the phylogenetic and phenetic relationships of phasmatids that occur in eastern Puerto Rico.

Materials and Methods

Walking sticks were collected in the Caribbean National Forest (18°10′N, 65° 30′W), Puerto Rico, between 12 June and 2 August 1985. Specific localities of collection and sample sizes (N) for the four taxa examined were: *Diapherodes achalus*, (a.) km 10.6 on route 186 (N = 1), El Yunque Quadrangle, Municipality of Naguabo; (b.) km 13.5 on route 191 (N = 4), El Yunque Quadrangle, Municipality of Naguabo; species X, km 13.5 on route 191 (N = 13), El Yunque Quadrangle, Municipality of Naguabo; *Lamponius portoricensis*, near route 180 (N = 66), El Verde Field Station, Municipality of Rio Grande; *Psuedobacteria yersiniana*, (a.) km 10.6 on route 186 (N = 13), El Yunque Quadrangle, Municipality of Naguabo; (b.) km 13.5 on route 191 (N = 5), El Yunque Quadrangle, Municipality of Naguabo. After collection, all individuals were transported to El Verde Field Station and were identified to specific level. Each specimen (minus abdomen) was placed in a 1.5 ml Eppendorf tube and immediately frozen; upon arrival at the Department of Biological Sciences, Texas Tech University, specimens were stored at −70°C.

Prior to allozymic analysis, each individual was homogenized in a buffered solution (pH = 6.8). The tissue homogenate was analyzed using standard horizontal starch-gel electrophoretic techniques (Selander et al. 1971, Harris and Hopkinson 1977). The following loci were examined: acid phosphatase (Ap); aldehyde oxidase (Ao); esterase-1, -2, and -3 (Es-1, -2, -3); glucose dehydrogenase (Gdh); glucose phosphate isomerase (Gpi); glutamate oxaloacetate transaminase-1 and -2 (Got-1, -2); leucine amino peptidase-1 and -2 (Lap-1, -2); malate dehydrogenase-1, -2, and -3 (Mdh-1, -2, -3); nucleoside phosphorylase (Np); peptidase-1 and -2 (Pep-B-1, -2); and phosphoglucomutase-1, -2, and -3 (Pgm-1, -2, -3). Only loci with consistent banding patterns (Ap, Es-1, Gdh, Lap-1, Mdh-1, -2, Pgm-1) were used in the subsequent analyses.

When multiple isozymes of a protein were present, the locus that migrated the farthest anodally was designated as "1," and loci

that migrated progressively in the direction of the cathode were given higher numerical designations. For each locus, the most common allele was designated as "100" and other alleles were assigned numeric values according to their mobility relative to the most common allele.

Genetic distances between each pair of taxa were calculated from allelic frequency data (Nei 1972, Rogers 1972). Nei's (1972) and Rogers' (1972) genetic distance values were similar, therefore, only Rogers' (1972) genetic distance values were used in subsequent analyses. Relationships among species X, *D. achalus*, *L. portoricensis*, and *P. yersiniana* were analyzed by genetic distances (Rogers 1972) and summarized in the form of a distance dendrogram that was obtained from a UPGMA (unweighted pair-group method using arithmetic averages; Sneath and Sokal 1973) clustering method. Phyletic relationships also were summarized in the form of an unrooted tree, produced by the Wagner parsimony analysis (Farris 1970) using the WAGNER78 package and the Fitch-Margoliash analysis of the distance matrix (Fitch and Margoliash 1967).

Results

Allele frequencies of the seven polymorphic loci and their distribution within taxa appear in Table 1. For the Ap locus, in which a total of seven alleles were detected, *D. achalus* was polymorphic for the two slowest alleles ("85" and "90"), species X was fixed for the "95" allele, and *L. portoricensis* was fixed for the "100" allele. *Pseudobacteria yersiniana* was extremely polymorphic in possessing the five fastest alleles ("95" through "110") at the Ap locus, thereby sharing the "95" allele with species X and the "100" allele with *L. portoricensis*. Only three alleles were detected at the Es-1 locus. *Diapherodes achalus*, species X, and *P. yersiniana* were each fixed for the "100" allele, whereas *L. portoricensis* was polymorphic and possessed all three alleles. No fixed differences or unique distribution of alleles was detected for either Gdh or Lap-1, with the exception that *P. yersiniana* possessed a greater number of alleles than any other taxon examined for these loci. For Mdh-1, *D. achalus* and species X shared two of the six alleles ("95" and "100" alleles) and each had approximately the same frequency in each taxon. *Lamponius portoricensis* was fixed at this locus for the "100" allele, which also was detected in *D. achalus* and species X. Again, *P. yersiniana* exhibited a high degree of polymorphism by possessing all six of the alleles for Mdh-1. For Mdh-2, only *D. achalus* was fixed for an allele ("100" allele), whereas the other three taxa were each characterized by the presence of all three alleles. Finally, for Pgm-1, only *L. portoricensis* was fixed for an allele ("100" allele), whereas the other three taxa each exhibited much polymorphism. Rogers' (1972) genetic distance values between *D. achalus* and species X, *D. achalus* and *L. portoricensis*, and *D. achalus* and *P. yersiniana* were 0.349, 0.524, and 0.475, respectively. Distance values between species X and *L. portoricensis*, species X and *P. yersiniana*, and *L. portoricensis* and *P. yersiniana* were 0.455, 0.426, and 0.571, respectively. Genetic distance relationships among the four taxa of walking sticks are summarized in the form of a distance dendrogram (Fig. 1).

Based on a phenetic analysis of allozymic data, species X is more similar to *D. achalus* than to *L. portoricensis* or *P. yersiniana*. Both the Wagner and Fitch-Margoliash analyses for phyletic relationships gave identical tree topologies; therefore, only the result of the Fitch-Margoliash analysis is presented. Phyletic relationships, summarized by the Fitch-Margoliash analysis were generated from an unrooted tree that reflects the actual observed genetic distance in the length of the branches (Fig. 2). The average value of heterozygosity (H) for these four taxa is 0.060. Heterozygosity estimates for each taxon are: $H = 0.051$ (species X), $H =$

Table 1. Allele frequencies of seven variable loci for four taxa of walking sticks collected from the Caribbean National Forest (18°10′N, 65°30′W), Puerto Rico. See text for locus abbreviations. The common allele is designated as the "100" allele and additional alleles are numbered according to the mobility of their products relative to that of the common allele. Alleles not listed in the table are: Ap-110, Es-1-95, Gdh-95, Mdh-1-115, Mdh-2-115, and Pgm-1-120 (their frequencies can be obtained by subtraction).

Locus	Allele	*D. achalus*	Species X	*L. portoricensis*	*P. yersiniana*
Ap	105	0.000	0.000	0.000	0.267
	100	0.000	0.000	1.000	0.233
	98	0.000	0.000	0.000	0.367
	95	0.000	1.000	0.000	0.100
	90	0.400	0.000	0.000	0.000
	85	0.600	0.000	0.000	0.000
Es-1	105	0.000	0.000	0.562	0.000
	100	1.000	1.000	0.308	1.000
Gdh	115	0.400	0.143	0.000	0.067
	112	0.000	0.000	0.000	0.133
	110	0.000	0.000	0.000	0.667
	105	0.200	0.214	0.175	0.067
	100	0.400	0.643	0.635	0.067
Lap-1	110	0.400	0.000	0.000	0.056
	105	0.600	0.286	0.063	0.000
	100	0.000	0.500	0.813	0.167
	95	0.000	0.215	0.125	0.778
Mdh-1	110	0.000	0.077	0.000	0.177
	100	0.500	0.462	1.000	0.118
	95	0.500	0.462	0.000	0.177
	90	0.000	0.000	0.000	0.441
	80	0.000	0.000	0.000	0.059
Mdh-2	110	0.000	0.357	0.273	0.177
	100	1.000	0.571	0.561	0.235
	95	0.000	0.071	0.167	0.529
Pgm	110	0.100	0.214	0.000	0.250
	105	0.100	0.286	0.000	0.056
	100	0.800	0.393	1.000	0.583
	98	0.000	0.036	0.000	0.000
	95	0.000	0.000	0.000	0.028
	85	0.000	0.000	0.000	0.056
	75	0.000	0.071	0.000	0.000

0.057 (*D. achalus*), $H = 0.044$ (*L. portoricensis*), and $H = 0.087$ (*P. yersiniana*).

Discussion

Rogers' genetic distance values indicate that considerable genetic divergence has occurred among the four taxa examined. Values range from 0.349 for the pairwise comparison of species X and *D. achalus* to 0.571 for the pairwise comparison of *L. portoricensis* and *P. yersiniana*. Nevo (1978) reported levels of heterozygosity for a variety of invertebrates; however he did not report data for the Phasmatidae. The heterozygosity values in this study occur within the range of those reported for closely related Orthoptera and other insects.

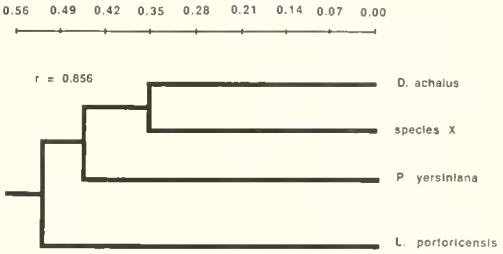

Fig. 1. Dendrogram that depicts the relationship of *Diapherodes achalus, Lamponius portoricensis, Pseudobacteria yersiniana,* and an unnamed taxon (species X) derived from a UPGMA of Rogers' genetic distance coefficients.

The limited research that has been reported on stick-insects from Puerto Rico has focused on taxonomy (Gray 1835, Burmeister 1838, Saussure 1868, Brunner and Redtenbacher 1892, Rehn 1903, Rehn and Hebard 1938, Wolcott 1923, 1936, 1941, 1948, Moxey 1971, 1972), systematics (Moxey 1972), population dynamics and natural history (Wolcott 1951, Willig et al. 1986), or effects of radiation (Virkki 1970). Based upon distributional data, Moxey (1972), suggested that the Antillean genera considered herein (*Diapherodes, Lamponius,* and *Pseudobacteria*) probably each evolved from Central American stocks, which subsequently invaded the West Indies from west to east. Moreover, he noted that *Lamponius* and *Diapherodes* probably evolved from a common stock. Our allozymic data provide limited support to the hypothesis that *L. portoricensis* and *P. yersiniana* may have evolved from different ancestral stocks in that they are separated by a genetic distance much greater than are the other taxa in the UPGMA (Fig. 1).

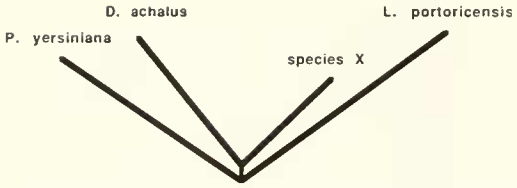

Fig. 2. Unrooted tree generated by a Fitch-Margoliash analysis based on Rogers' genetic distances for *Diapherodes achalus, Lamponius portoricensis, Pseudobacteria yersiniana* and an unnamed taxon (species X).

Nonetheless, the large genetic distance between all taxa in the UPGMA (Fig. 1) and the short internode distance in the Fitch-Margoliash tree (Fig. 2) fail to provide conclusive information about the systematic relationships of these taxa or the proper affiliation of species X. Clearly, the phylogenetic tree should be viewed with caution because it does not involve a monophyletic group and no out group was used to root the tree. Five possible phylogenies (we exclude those with unresolved trichotomies if rooting occurs at vertices) are congruent with the Fitch-Margoliash topology (Fig. 2). In three of them, species X is more closely related to *D. achalus* than to any of the other taxa. Alternatively, species X may be less related to any of the other taxa than those taxa are to each other, or *L. portoricensis* and *P. yersiniana,* as a group, may be more closely related to species X than any of those three taxa are to *D. achalus.*

Future studies addressing phasmatid systematics in the West Indies should obtain adequate samples of all taxa occurring throughout the West Indies, and include an outgroup by which one could root a phylogenetic tree. Thereafter, it would be possible to test a variety of hypotheses concerning phasmatid systematics and thereby facilitate the classification of species X as well.

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